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Variations of physicochemical properties of stingless bee honey from different botanical origin in state of Johor, Malaysia.

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Abstract. Physicochemical parameters analysis was used in the identification of varietal honey from different botanical origin. Botanical origin is one of the important parameter to be known as a quality control to meet the legislated mandatory requirement during commercialization. This study assessed the influence of the botanical origin on the physicochemical properties of stingless bee honey or known as *kelulut* honey in state of Johor, Malaysia. The honey samples were collected in six different botanical origins namely acacia,coconut, mangrove, starfruit, multifruits and multiflowers produced by bee species of *Heterotrigona itama*. Tualang honey from bee species of *Apis dorsata* was included as a positive control. The physicochemical properties in terms of pH, moisture, ash content, electrical conductivity (EC) and colour intensity were measured. The result obtained revealed an average pH (3.40 ± 0.15), moisture (28.9 ± 2.69 g/100g), ash (0.37 ± 0.27 g/100g), electrical conductivity (0.11 ± 0.07) mS/cm, and colour intensity (692 ± 187) mAU. In general, the raw honey from state of Johor presented satisfactory quality according to the result obtained help in honey identification and authenticity as well as to sustain meliponiculture industry in Malaysia.

Keywords. Stingless Bee Honey; Meliponiculture; Botanical Origin; Physicochemical Properties.

1. Introduction

Among the abundant species of stingless bee all over the world, the *trigona* sp. otherwise known as *lebah kelulut* is a stingless bee species commonly found in Malaysia. *Heterotrigona itama* is the most

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abundant species found in Southern part of Malaysia [1]. Recently, the increasing demand of stingless bee products such honey, propolis and pollen has attracted many entrepreneur to get involved in meliponiculture activity. In moliponiculture, it is easier to construct an artificial hive that will maintain, propagate and utilize the colony and increase the amount of honey production when compare to the honey bees, which regularly collapse and is more expose to contamination. The variation of beekeeping management in developing the beehives, selecting stingless bee species, preparing the plant sources and geographical origin might produce its own unique type of bee product which differ from one another.

The botanical sources of honey has been reported to have significant effect of the physical and chemical characteristics [2]. Physicochemical properties of honey is a quality parameter to be known. Quality control of honey is essential in determining its suitability for processing into marketable product. The studies on physicochemical properties of honey produce by stingless bee have been reported in several countries such as South America, Venezuela, Columbia, Brazil and Argentina [3]. Previous study also reported that some physicochemical composition of Malaysian honeys such moisture content and sugar profile did not meet the quality standard set by the International Honey Commision (IHC) [4]. Hence, the latest Malaysian stingless bee honey quality standards must be practiced and implemented in order to control the local honey quality and authenticity.

Thus, the objective of the present study is to assess the influence of the botanical origin on the physicochemical properties of stingless bee honey produced by *H.itama* species which are widely used in commercial beekeeping in state of Johor, Malaysia.

2. Material and methods

2.1. Honey samples

Honey samples from the beehives produced by *H.itama* species were taken during field trips from October to December 2017. Six honey sample from different botanical origins namely acacia, coconut, mangrove, starfruit, multifruit, and multiflowers were investigated (Table 1). Tualang honey produced by bee species of *Apis dorsata* used as positive control. Samples were stored in sterilized amber glass containers, shipped to the laboratory, and then maintained at 4^0C in the dark until analysis.

Table 1. The identification of honey samples

Sample code	Botanical origin	Production region
K01	Acacia	Kota Tinggi, Johor
K02	Coconut	Tangkak, Johor
K03	Mangrove	Batu Pahat, Johor
K04	Starfruit	Muar, Johor
K05	Multifruits	Kulai, Johor
K06	Multiflowers	Batu Pahat, Johor
K07	Tualang	Tangkak, Johor

2.2. Physicochemical analysis

The physicochemical parameters of honey produced by stingless bee were assessed following the Malaysian Standards (2017), method of analysis described by AOAC (2006) and International Honey Commission (2009). Among, the parameters tested are moisture (g/100g), ash (g/100g), electrical conductivity (mS/cm), pH, and colour intensity (mAU). All the experiments were carried out in triplicate.

The oven method previously described by AOAC 925.45 was used to measure moisture content [5]. About 5 grams of honey sample was placed in a stainless steel flat dish with tight-fit-cover and

heated for 24 hours at 105^0C in a convention oven. Moisture content was determine in the unit of g/100g honey.

Electrical conductivity of the honey sample was measured following the method demonstrated by International Honey Commission [6] as measured in a 20% (w/v) solution of honey sample in deionized water using a ENA Series Network Analyzer model E5071C. The impedance data were measured and the electrical conductivity was analyzed using nyquist plot.

Colour intensity of honey sample was measure using the spectrophotometry method [7]. Briefly, honey was diluted with warm water (45-50^0C) to 50% (w/v) and the solution was filtered to remove all coarse particles. Thereafter, the sample absorbance was measured using a spectrophotometer (T60 UV-VIS spectrophotometer) at 450 and 720 nm, and the difference in absorbance was expressed as mili absorbance unit (mAU).

The pH of honey sample was measure according to AOAC method 962.19 [8]. Briefly 10 grams of sample was dissolved in 75 ml of deionized water and a digital pH meter was used to measure pH at room temperature (Oakton pH 700 Benchtop Meter).

The ash content was measured based on AOAC method 920.181 by placing a crucible in an oven at 100^0Cfor 1 hour. After cooling, 10 grams of honey was placed into the crucible and was heated in a Muffle furnace at 500^0C for 3 h and then reweighed. Ash percentage was calculated.

2.3. Statistical analysis

The analyses were perform in triplicate and the results were reported as mean ± standard deviation (SD). The data obtained in the study was analyzed statistically using a one-way analysis of variance (ANOVA) followed by post-hoc Tukey's test. All statistical analyses were performed using IBM SPSS Statistics 22 and the significant differences were statistically considered at $p \leq 0.05$.

3. Result and discussion

3.1. Physicochemical properties of honey

The range of value, mean and standard deviations of moisture content, pH, ash content, electrical conductivity and colour intensity are listed in Table 2. The pH was found to be the most consistent parameter showing the least variability measured. Honey is acidic in natural as it is affected by the storage and circumstances of extraction, influencing the texture, stability and shelf-life of final product. This is reflected in their low pH value range from 3.17-3.46 ($p \leq 0.05$) among the honey sample collected from different botanical sources produced by same bee species. The low pH of honey is at least partly due to the presence of gluconic acid [9]. Acacia honey was the most acidic followed by mangrove, coconut, multiflower, multifruit and starfruit honey. The result obtained is close to those previous reported for local pH value ranging from 3.24 to 3.44 [10], Brazilian stingless bee honeys has pH ranging from 2.90 to 3.38 [11] and Thai stingless bee honey is from 3.10 to 3.90 [12]. Generally, honey from a floral source or honeydew honey displayed high acidic pH values with no direct relationship with the botanical origin. However, the pH have been linked by other parameters that are affected by botanical and geographical origin such as mineral content [13].

Honey from stingless bee has always been characterized to possess higher moisture content than most honey from sting bee. Water content has been associated with the botanical sources, although soil, climatic conditions, period of collection as well as processing aspects may affect this parameter [14]. All tests samples shown the average moisture content is 28.9 ± 2.69 g/100g with the range from 27.0 to 31.0 g/100g ($p \leq 0.05$) . This is much higher as compared to the tualang honey produced by *Apis dorsata* (23.4 g/100g). Comparable data from state of north-eastern Brazil showed similar range of moisture (22-35 g/100g) [15]. The moisture values found in this study comply with the Malaysian standards which indicate that the analyzed samples presented a good quality.

Colour is one of the physical property observed immediately by the consumer is determined in relation to the botanical origin, minerals, phenolic content, storage time and temperature [16]. Previous studies on stingless bee honey often neglected any measure of honey's colour. The data from our

analysis show values ranging from 408 mAU to 978 mAU. There is significant different ($p \leq 0.05$) in term of colour analysis between all honey samples. The value of colour intensity were similar to the previous report of local honey ranging from 500 to 1200 mAU [17]. The result obtained in this current study is in line with that obtained from Indian and Romanian honeys which were in the range of 524 to 1678 mAU and 210 to 1228 mAU respectively [18,19]. Among the physicochemical properties evaluated, colour intensity is the only parameters that is highly correlated with the phenolic content as well as antioxidant properties of honey. Indeed, darker coloured honey contains higher total phenolic content leading to a suggestion that the honey can have a high bioactivity. Besides, the phenolic profiles of honey and their antioxidant capacity depends on the botanical origin visited by the bee [20].

The electrical conductivity has been defined as a measure of the quantity of mineral elements contained in honey [21]. The study showed the value of the electrical conductivity (EC) of the honey ranged from 0.04 to 0.22 mS/cm, which are within the Codex standard limit of < 0.80 mS/cm [22]. The average electrical conductivity of all samples is 0.1 ± 0.07 mS/cm. ANOVA analysis indicated that there are significant differences between the honey samples in term of EC ($p \leq 0.05$). This is comparable to the report of electrical conductivity of honey from predominant sources produced by different stingless bee spices in Brazil ranging from 0.15 to 1.34 mS/cm [23]. A study from Thailand reported the electrical conductivity of honey produced by *Apis mellifera* to be 0.26 ± 0.04 mS/cm, which is higher than our finding. The electrical conductivity is correlates well with the ash content of honey. Others factors, including floral sources, quantity of proteins and organic acids, and storage duration can also affect the electrical conductivity of honey samples [4].

Our result showed that the ash content ranged between 0.14 to 0.89 g/100g ($p \leq 0.05$). All stingless bee honey examined have ash contents within the suggested by Malaysian standards (max 1.0 g/100g). The values were almost similar to Suntiparapop's report where the ash content for *Tetragonula laeviceps* collected from two adjacent provinces of Thailand with 0.20 g/100g from Trat province and 0.33 g/100g from Chanthaburi province [24]. The ash content of honey represents its total mineral present which is directly related to the geographical, environmental, and botanical aspects of it origin [25].

Table 2. Physicochemical characteristics of *Kelulut* honey from *H.itama* species

Botanical origin	Sample code	Moisture (g/100g)	Electrical conductivity (mS/cm)	Ash (g/100g)	pH	Colour intensity (mAU)
Acacia	K01	$30.67^{b} \pm 0.58$	$0.06^{d} \pm 0.00$	$0.22^{ij} \pm 0.07$	$3.17^{m} \pm 0.08$	$408^{q} \pm 3.61$
Coconut	K02	$29.33^{b} \pm 2.52$	$0.22^{h} \pm 0.00$	$0.89^{l} \pm 0.06$	$3.38^{no} \pm 0.06$	$673^{s} \pm 1.73$
Mangrove	K03	$29.67^{b} \pm 1.15$	$0.11^{e} \pm 0.01$	$0.42^{jk} \pm 0.07$	$3.32^{n} \pm 0.03$	$978^{v} \pm 5.00$
Starfruit	K04	$31.00^{b} \pm 1.00$	$0.04^{cd} \pm 0.02$	$0.16^{ij} \pm 0.05$	$3.46^{o} \pm 0.03$	$499^{r} \pm 1.73$
Multifruits	K05	$27.00^{ab} \pm 1.00$	$0.04^{c} \pm 0.01$	$0.14^{i} \pm 0.02$	$3.44^{o} \pm 0.03$	$737^{t} \pm 3.46$
Multiflowers	K06	$30.67^{b} \pm 2.52$	$0.13^{f} \pm 0.04$	$0.54^{k} \pm 0.12$	$3.38^{no} \pm 0.02$	$681^{s} \pm 6.00$
Tualang	K07	$23.33^{a} \pm 1.53$	$0.17^{g} \pm 0.12$	$0.23^{ij} \pm 0.05$	$3.68^{p} \pm 0.02$	$869^{u} \pm 4.58$

Note: Values expressed as mean ± standard deviations. $^{a\text{-}v}$=Different superscript letters in the same column indicate significant differences (ANOVA, $p \leq 0.05$) between honey samples from different botanical origin produced by same bee species according to Tukey's test.

4. Conclusion

The botanical origin of the six types of honey samples analysed have influenced their physicochemical parameters. In regards to the parameters that guarantee the quality of honey, all tested honey met the criteria proposed by the Malaysian standards. The honey obtained from different botanical origin has a significant effect of the physicochemical characteristics. Finally,the understanding of physicochemical properties of stingless bee honey is very vital in order to set up certification marks and improve local beekeeping, as well as the potential for possible export.

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